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# An Experimental Model for Bubble Formation in Diving Seals and Porpoises

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## LONG-TERM GOALS

Understanding the behavior of gas within marine mammals as pressure varies with depth is critical for models of gas management in diving mammals. Interpretation of such data allows a mechanistic understanding of the effects, or absence of effects, of acoustic stressors on diving marine mammals.

## OBJECTIVES

1. How does lung collapse progress in diving mammals with increasing depth?
2. Where residual air remains, what is the air to blood transfusion distance and rate?
3. How and why do gas bubbles develop in bycaught seals and porpoises drowned at various depths?
4. What are the gas compositions of bubbles detected in marine mammals?
5. Can ultrasound detect bubbles in intact marine mammals?

# Report Documentation Page

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14. ABSTRACT
**Understanding the behavior of gas within marine mammals as pressure varies with depth is critical for models of gas management in diving mammals. Interpretation of such data allows a mechanistic understanding of the effects, or absence of effects, of acoustic stressors on diving marine mammals**

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## APPROACH

### Specific Aims Part A (CT etc.)

1. Measurement of cadaver lung capacity and collapse in laboratory: CT scans will be made, at the surface and in pressures equivalent to up to 200m depth, in cadavers in a carbon fiber, water filled, pressure vessel.
2. Histological analysis of air to blood vessel distances in sinuses and trachea and wherever else residual gas is shown to remain in Aim 1.
3. Gas bubble analysis in necropsy samples from bycaught and other stranded cases.
4. Serial CT scans of bubbled bycaught cadavers to understand the dynamics of post mortem bubbling and to compare bubble formation risk with lipid content of different tissues.

Aims 1 through 4 will substantiate or improve assumptions in current models of breath hold diving and the effects of acoustic stressors thereon.

### Specific Aims Part B (Ultrasound)

1. Development of a bubble detection protocol with a portable ultrasound unit using bycatch known to be bubbled by CT, prior to necropsy. Such a protocol would substantially enhance evaluation of the presence or absence of bubbles in mortality events.

## WORK COMPLETED

The major goal for the CT portion in this project in the first of this two year project was to design and build a hyperbaric CT chamber. This required substantial research into potential engineering and fabrication solutions, and dove-tailing the product to the available Siemens CT scanner in the Ketten Laboratory at WHOI. A commercially available fiberglass pipe and flange system (Green Thread 250, Fiberglass Systems, Little Rock, AR) was found to be adequately non-attenuating. A rail bed and cart for the chamber was fabricated at WHOI incorporating low friction cars and tracks (Harken USA, Peewaukee, WI). The scanner table drive had insufficient torque to advance the loaded chamber. Therefore a motor drive system was built (Artec Imaging, Cornelius, NC) that was magnetically coupled to the movement pattern of the CT scanner table, without exceeding the weight capacity of the table (450lb). The weight of the loaded hyperbaric vessel was 3,068lb.

We are developing a protocol to deploy freshly dead seal and dolphin carcasses in the HCT chamber. The carcass is currently secured in the chamber within a rack system, after a tracheotomy tube with valves is tied into the trachea (Figure 1). This allows controlled inflation of the lungs to a predetermined volume prior to the experiment. The chamber is then filled with water, while tilted to allow as much air to be expelled from the chamber as possible. It is then pressurized to the highest test pressure – currently about135psi (Figure 2). The chamber has a 250psi rating. The system is then scanned at the starting pressure, and the pressure is reduced to half the starting pressure, scanned again, and then released to ambient and scanned again (Figures 3-6). During this process as the buoyancy changes the animal tends to shift position and twist. The next step is to test a suction cup system to better restrain the cadaver. In addition the motor drive only positively affects one side of the cart. A linkage to a second drive belt is being installed that will smooth the travel of the system by driving the cart symmetrically.

The major goal for the ultrasound portion of the project in the first year was to research and acquire a portable ultrasound unit that would be suitable for deployment at beach strandings. 12MHz and 5MHz broadband transducers and a portable ultrasound system (Terason 3000, Teratech Corporation, Burlington, MA) were acquired. This system was tested on drowned bycatch dolphins and seals shown to be bubbled and not bubbled using CT. Bubbles, identified by a characteristic artifact, were found to be detectable in liver, kidney, blubber, muscle and eye using ultrasound. The system was then deployed during live mass strandings of dolphins, where bubbles were also detected.

## RESULTS

CT protocols were employed to image excised and *in situ* lungs, both in and out of the chamber. There was no loss of tissue detail or resolution for images in the pressure chamber. In this way the specimen can be scanned using helical data acquisition within the chamber at a range of pressures (Figure 7). Detailed analysis of the volume changes observed will be undertaken by a related project by Dr's Ketten and Fahlman once the mechanics of the system have been optimized. Preliminary estimates of pressure induced volume and CT attenuation change in a dolphin and a seal are shown in Tables 1 and 2. The attenuation value (Hounsfield units, HU) decreases as the air content in the lung increases. Preliminary measurements were made by manually drawing regions of interest (ROI) around the lungs using Osirix software (open source software available as a free download at http://www.osirix-viewer.com).

***Table 1 - Summary of pressure induced changes in volume and attenuation in a common dolphin (CCSN 06-064Dd)***

| | Left Lung | | Right Lung | |
|---|---|---|---|---|
| Pressure | Volume | Attenuation | Volume | Attenuation |
| 0 | 2823 | -801 | 1808 | -645 |
| 75 | 800 | -514 | 530 | -62 |
| 135 | 568 | -279 | 461 | 7 |

Units: Pressure = psi; Volume = ml; Attenuation = Hounsfield Units

***Table 2 - Summary of pressure induced changes in volume and attenuation in a grey seal (DO7450Hg)***

| | Left Lung | | Right Lung | |
|---|---|---|---|---|
| Pressure | Volume | Attenuation | Volume | Attenuation |
| 0 | 2577 | -599 | 1013 | -95 |
| 67 | 992 | -80 | 431 | 61 |
| 142 | 869 | 28 | 313 | 70 |

Units: Pressure = psi; Volume = ml; Attenuation = Hounsfield Units

In both cases the animal twisted so that the left side was uppermost, hence the left lung inflated faster than the right on pressure reduction.

B-mode ultrasound was evaluated as a diagnostic imaging modality for gas bubble detection in bycatch and dead stranded marine mammals (Figure 7). Topographic landmarks were established (Figure 8). For liver, the angle formed by a line from the eye to the umbilicus to the caudal insertion of the dorsal fin located the caudal tip of the lobe that is not shielded by ribs. The liver could also be imaged intercostally by moving the transducer in a cranial direction once the caudal part of the right lateral lobe had been identified. For kidneys, straddling a line dropped down from the caudal insertion of the dorsal fin, intersected with a line drawn from the eye to the lateral insertion of the peduncle, along the curve of the body, allows imaging of the kidney (Figure 8).

Findings were correlated with computed tomography (CT) findings and gross necropsy observations. Animals from 30lb through 1600lb were examined using the system. The eye, liver, kidneys and blubber-muscle interface were determined as structures that: 1) were easily and repeatedly identifiable, 2) were easily accessible and 3) were frequently gas bubbled when gas bubbling was present. Bubbles were found predominantly in bycaught animals drowned at depth (Figure 9). However, a limited ultrasound examination including the liver, kidneys and the blubber-muscle interface over these structures was then performed on live stranded marine mammals. No evidence of gas bubbling was identified in stranded grey seal neonates. Conversely, in two mass-stranding events involving four common and one whitesided dolphins, bilateral renal gas bubbling was identified in all animals (Figure 10).

## IMPACT/APPLICATIONS

Quantification of the change in volume of the various gas-filled structures allows an absolute measurement of the compliance of various critical structures. The resulting data will populate a new generation of mathematical models for determining how marine mammal lung tissues respond to pressure related gaseous changes in dissolved and gas phases during deep dives in collaboration with Andreas Fahlman and Darlene Ketten. Our understanding of the mechanics of the compression and collapse of the respiratory system is still rudimentary. The implied depths of lung collapse are highly variable and most likely vary with the diving lung volume. A better understanding of the relationship amongst pressure, alveolar volume, and the depth at which gas exchange ceases has important implications for understanding how diving mammals manage gases during diving.

If one assumes a rigid trachea and an infinitely compliant rib cage and lung, one can estimate the volume of the respiratory system to the point of lung collapse as:
$V_L = V_{L_o} \bullet (P_{amb_o} \bullet P_{amb}^{-1})$ where $V_L$ is the volume of the respiratory system at pressure $P_{amb}$, $V_{L_o}$ the initial diving lung volume and $P_{amb_o}$ the pressure at the surface. In the dolphin, $V_{L_o}$ was 4.6 l, and estimated $V_L$ would therefore be 772 ml at a pressure of 75 psi and 463 ml at 135 psi. The observed respiratory volumes at these pressures were significantly larger, agreeing with the suggestion that the structural pressure of the respiratory system may resist compression (Bostrom et al 2008). Alternatively, extremely high pressure differences should develop across the lung. Large pressure differences could result in pulmonary barotrauma, commonly called the squeeze in human breath-hold divers. In humans, redistribution of blood from the periphery into the thoracic cavity has been shown to help prevent elevated pressure differences by displacing the air volume. It is possible that a similar redistribution of blood occurs in live marine mammals which may help prevent lung squeeze. It is also possible that differences in the cytoarchitecture allow for higher transmural pressures to develop before barotrauma occurs.

The ultrasound study demonstrates that ultrasound may be used to identify the presence of some gas bubbles in marine mammals in the field. Furthermore, these findings document gas bubbling in live stranded animals and suggest that under normal circumstances marine mammals have physiologic and probably behavioral adaptations to avoid acute clinical decompression sickness.

## TRANSITIONS

We anticipate that the ability to image substantive biological material under different pressures will have a diverse range of academic and industrial applications once the method has been published.

## RELATED PROJECTS

Dr's Ketten and Fahlman will be analyzing hyperbaric scans to generate estimated probable compliance value changes for population of diving physiology models (Fahlman et al 2009).

## REFERENCES

Bostrom BL, Fahlman A, Jones DR. Tracheal compression delays alveolar collapse during deep diving in marine mammals. . *Respir Physiol Neurobiol* 2008;161:298–305.

Fahlman A, Hooker SK, Olszowka A, Bostrom BL, Jones DR (2009) Estimating the effect of lung collapse and pulmonary shunt on gas exchange during breath-hold diving: The Scholander and Kooyman legacy. *Respiratory Physiology & Neurobiology* 165:28-39

## PUBLICATIONS

Moore MJ, Bogomolni AL, Dennison SE, Early G, Garner MM, Hayward BA, Lentell BJ, Rotstein DS (2009) Gas bubbles in seals, dolphins and porpoises entangled and drowned at depth in gill nets. *Veterinary Pathology* 46:536-547

## PATENTS

None

## HONORS/AWARDS/PRIZES

None

***Figures 1 through 6 photographed by Tom Kleindinst, WHOI***

***Figure 1 – A tracheotomy is performed on a dead seal to tie in a valve system to allow inflation of lungs with a known volume of air.***


***Figure 2 – Cadaver is placed in pressure chamber, and end cap bolts torqued to 100lbf. Chamber is then filled with water at a tilt to expel all air, and then pressurized to chosen pressure (~140psi), using a manual hydraulic pump. The amount of water required to reach this pressure is measured with a sight gauge.***

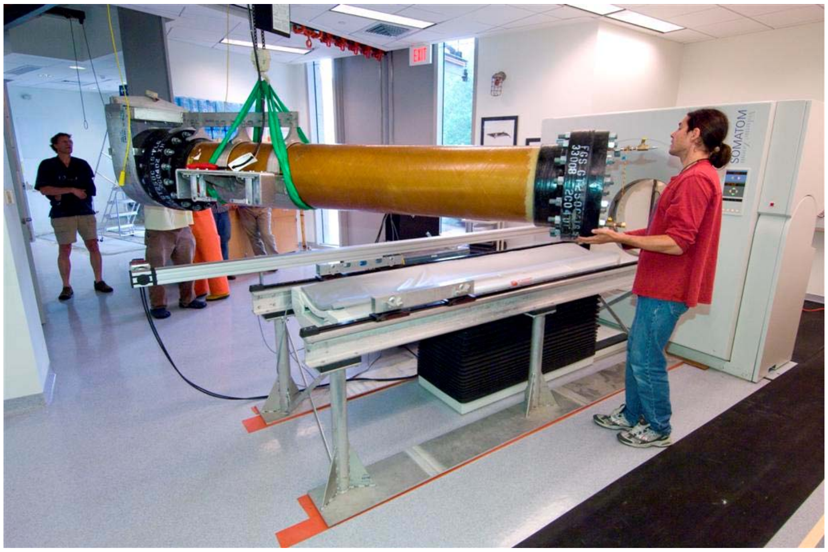

***Figure 3 – Chamber is transferred to CT scanner with an overhead crane. It weighs 3068lbs when loaded. To balance over the cart, a lead counterweight basket can be seen at left. CT scanner table is the grey bed between the two black rails upon which runs the cart system.***

***Figure 4 – Chamber attached to cart system on weight bearing rails, magnetically coupled to the CT scanner table. Control system for cart and coupler is black box to left.***

***Figure 5 – Chamber advanced through CT scanner.***

***Figure 6 – View of hyperbaric chamber on scanner bed from control desk.***

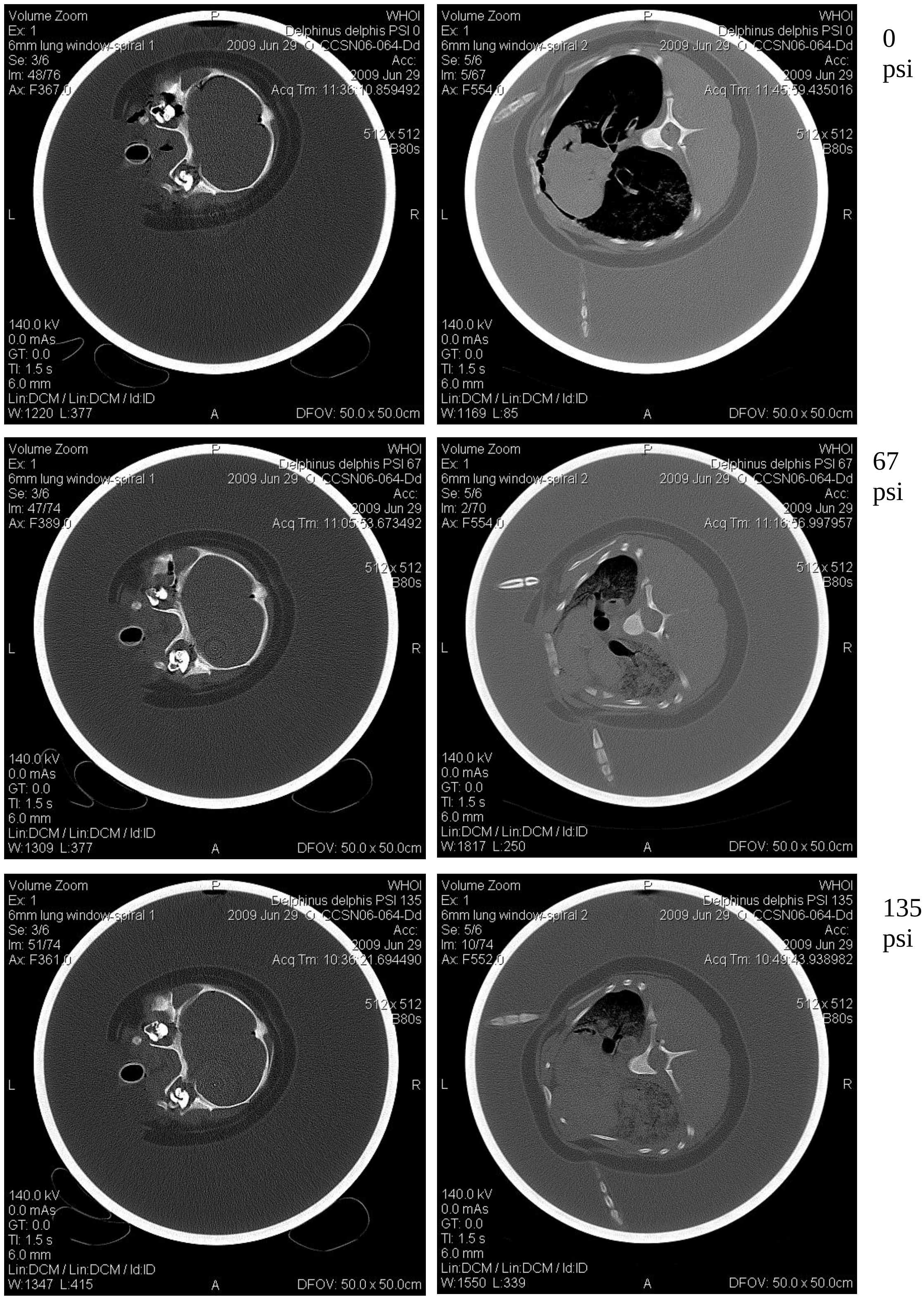


***Figure 7 – CT sections though the pressure chamber containing a common dolphin. Note lung compression (shrinking dark areas on right panel), whereas trachea remains the same size (dark circle on left series). Note also loss of buoyancy with increasing pressure.***

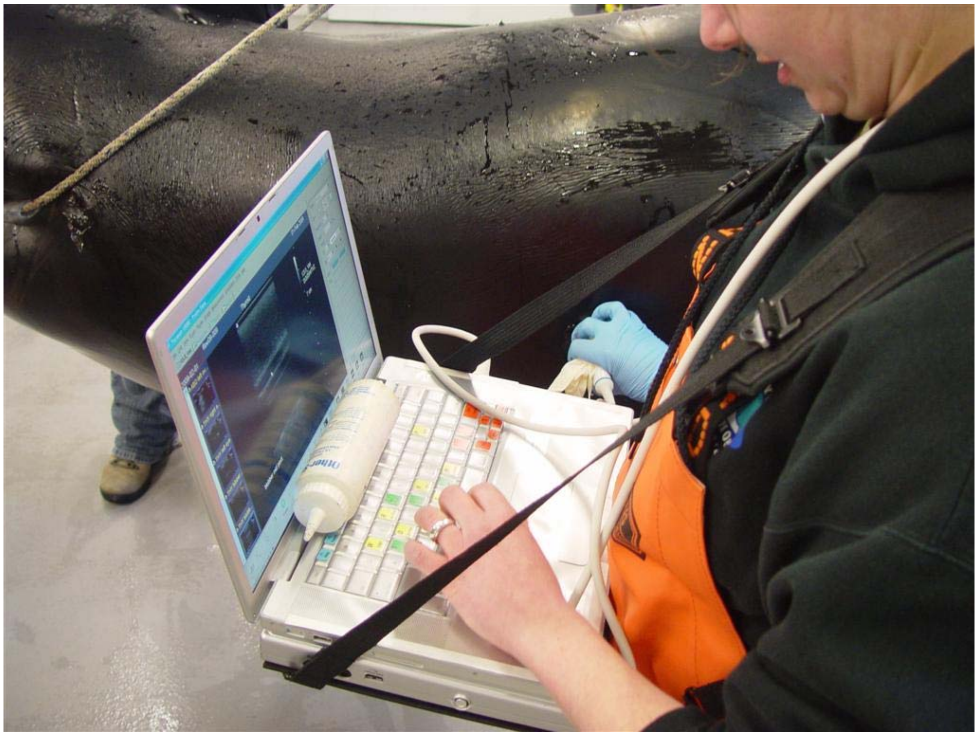

*Figure 8 – Terrason 3000 ultrasound system deployed on a single stranded pilot whale.*

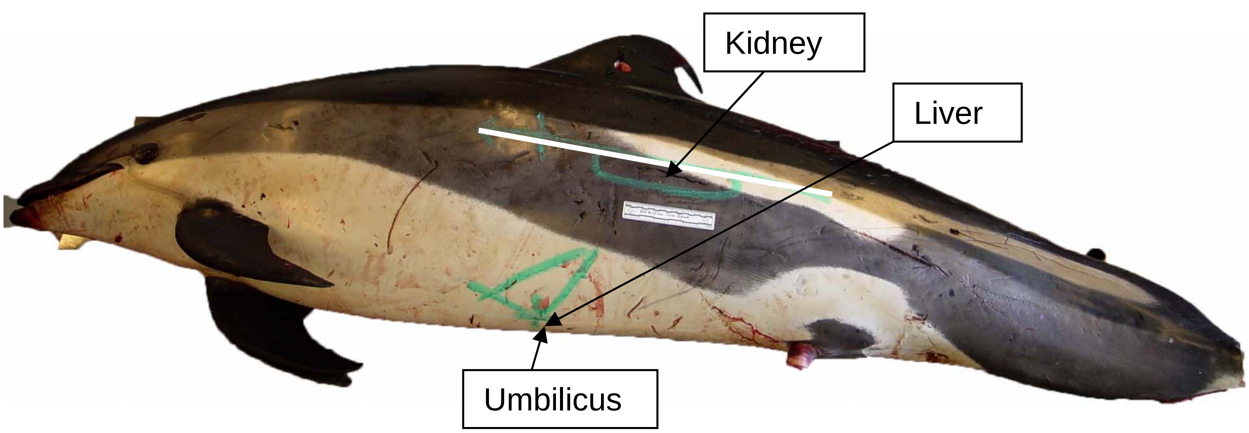


*Figure 9 – Topographic anatomy of a white sided dolphin showing how a line from the eye to the umbilicus to the caudal insertion of the dorsal fin locates liver and kidney for ultrasound exam.*

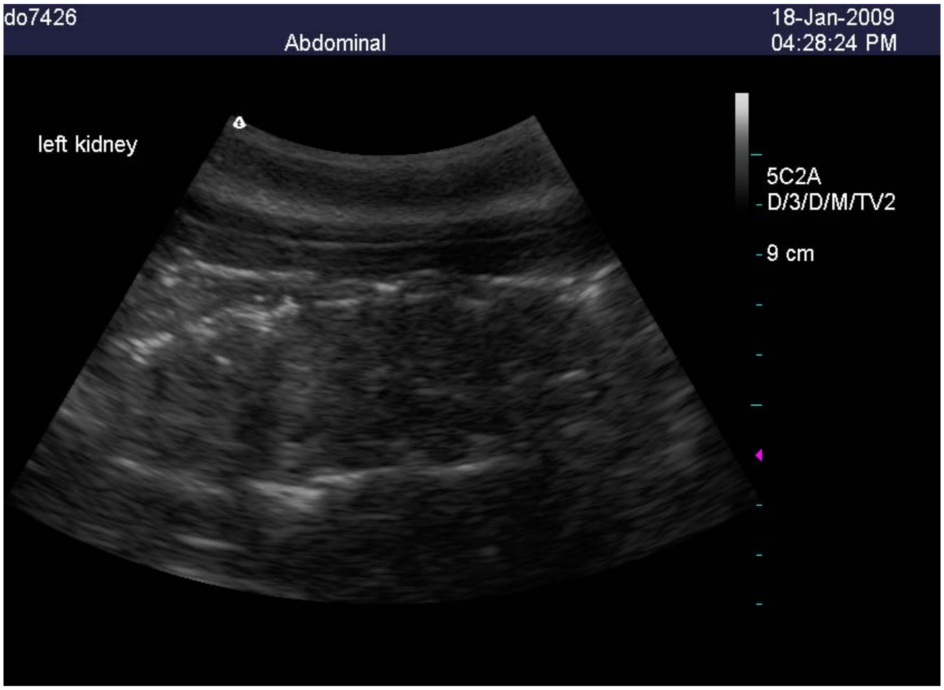


***Figure 10 – Bubbles detected by ultrasound by identification of the characteristic ring-down reverberation artifact in the capsular veins of the left kidney of a bycaught grey seal. Bubbles were confirmed grossly at subsequent necropsy***

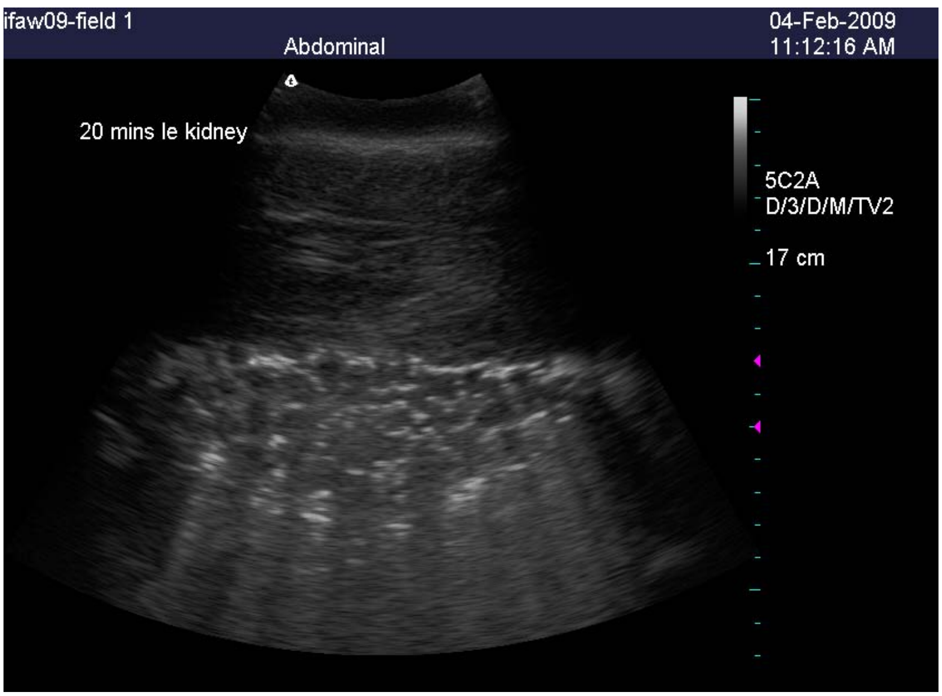


***Figure 11 – Bubbles detected by ultrasound in the kidney of a live stranded common dolphin.***